BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

II. REMARKS

Upon entry of the amendment, claims 1 to 15 and 64 to 68 will be pending. A marked version showing the amendments to the specification and the claims is attached as Exhibit A.

A. Regarding the Amendments

The specification has been amended to correct a typographical error, wherein a second occurrence of the abbreviation "GDF-8" was deleted. As such, the amendment does not add new matter.

Pursuant to the restriction requirement, claims 16 to 53 are cancelled herein without disclaimer, and without prejudice to Applicants' pursuing prosecution of subject matter encompassed within one or more of the claims in an application claiming the benefit of priority of the subject application.

Claim 1 has been amended to more clearly indicate that a transgenic aquatic organism of the invention comprises an insertion of a transgene "into an endogenous GDF-8 gene". The amendment is supported, for example, at page 5, lines 9-11, and, therefore, does not add new matter.

Claim 4 has been amended to indicate that the transgene comprises "a selectable marker sequence". The amendment was necessitated by the amendment to claim 1, which previously encompassed transgenes such as an antisense nucleotide sequence, which are the subject matter of new claim 64. The amendment to claim 4 is supported by claim 8 as originally filed and, for example, at page 52, lines 7-9. As such, the amendment to claim 4 does not add new matter.

Claim 8 has been amended to correct a typographical error, and to correct an informality, wherein there was no antecedent basis for the term "endogenous GDF-8 gene." As such, the amendments do not add new matter.

Claim 13, which previously depended from claim 8, has been amended to depend from claim 7. The amendment is supported, for example, at page 49, lines 7-10, and, therefore, does not add new matter.

New claims 64 to 68 have been added. The new claims are based on claims 1, 2, 3, 5 and 6, respectively, and are further supported, for example, at page 5, lines 7-11; and page 49, lines 6-19. As such, the newly added claims do not add new matter.

B. Restriction Requirement and Species Election

In the Restriction Requirement mailed November 20, 2001, by a previous Examiner, there was no indication of the classes or sub-classes to support the division of the claims. Applicants' provisionally elected Group I in order to be responsive to the Action, and requested that class and sub-class information be provided to support the alleged undue burden that was alleged to be required to examine the various claims.

In the present Office Action, class and subclass information is provided. It is submitted, however, that the information appears to be incomplete and, in some cases, does not appear to be support the division of the claims. For example, the claims of Groups I and II recite the same class 800 and subclasses. Group II also recites class 514, which also is applicable to Group I. In addition, it is unclear which "subclasses" should be associated with which class (Group II recites, "class 800, 514, subclass 21+, 4+ and 44+, for example"), although it is noted that most of the combinations of classes and subclasses appear to be equally relevant to Groups I and II. Applicants are uncertain as to the meaning of the "plus" sign, as no such indication was found in the "Manual of Patent Classification" on the U.S. PTO web site. Similarly, Groups IV and VI both recite class 530, subclass 300, which also is included in Group V.

As such, it is submitted that the evidence of record does not appear to support the position that the claims of Groups I and II should be divided, or that the claims of Groups IV and VI and, arguably, Group V, should be divided. Although it is maintained in the present Office Action that an undue burden would be required to search, for example, the claims of Groups I and II, the classification as set out by the Examiner indicates that the same information would be searched for Groups I and II. As such, there is nothing of record to indicate that it would be an undue burden to search the claims of Groups I and II together.

In order to advance prosecution, Applicants have cancelled the non-elected claims. However, it is again requested that the basis of the Restriction Requirement be set forth with specificity so as to provide an objective basis for arguing rejoinder of claims in a divisional application.

With respect to the species election, it is stated in the Office Action that the phenotype of transgenic animals of different species is unpredictable, particularly with respect to gene disruption. Applicants are uncertain, however, how the predictability or unpredictability of a result is relevant to the requirement to elect a species. It is further stated in the Office Action that the methods of creating the different species would comprise different method steps and, therefore, that the different species of aquatic animals do not share a commonality of operation, function or effect. However, there is no indication in the Office Action as to what steps may be different, whereas, as set forth in the previous response, it was pointed out that the transgenic organisms all share a common disruption of the GDF-8 gene, and the methods require disrupting the GDF-8 gene and identifying transgenic organisms.

With respect to the species election, it is noted that no art has been cited in the present Office Action against the provisionally elected species. Furthermore, even where references are cited in support of the enablement rejection, none is directed to an aquatic species (see, also, discussion below). As such, other than the fact that the organisms set forth in each of the two alleged "species" are recited in different claims, it is not clear why a species election is required, and there is no apparent reason why the various species cannot be examined together. In fact, support for the rejection of the claims as allegedly lacking enablement is based, in part, on the various different species of aquatic animals encompassed within the claims (see Office Action at page 10). As such, it is again requested that the species election be removed.

C. Regarding the Sequence Listing

As requested in the Office Action, a substitute CRF and paper copy of the Sequence Listing, along with the appropriate Statements, have been submitted under separate cover to Box Sequence Listing. Accordingly, it is respectfully requested that this objection be withdrawn.

D. Regarding the Drawings

The objection to the Drawings is acknowledged. Applicants will submit formal Drawings upon receiving an indication that the claims are in condition for allowance.

E. Rejections under 35 U.S.C. § 112

The objection to the specification and corresponding rejection of claims 1 to 15 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement are respectfully traversed.

1. State of the art regarding transgenic aquatic organisms

As an initial matter, it is noted that, while several references have been cited describing double muscling in mammals and the generation of transgenic mammals, no references directed to transgenic aquatic animals were cited. The absence of such references is remarkable in view of the well advanced stage of transgenic aquatic animal technology, and of the various commercial endeavors directed to such transgenic organisms. In this respect, it is noted that a search of google.com on the world wide web using the term "transgenic fish" or "transgenic salmon" revealed numerous references relating to transgenic aquatic organisms, a few examples of which are provided as Exhibits B, C, D and E.

For example, a report by the Environmental Media Services, updated October 2000, indicated that about 20 varieties of finfish and shellfish have been genetically engineered, including salmon, catfish, carp, pike, shrimp, striped bass, and shellfish, typically with genes that speed growth (see Exhibit B). Exhibit A specifically refers to two companies that are awaiting FDA approval to sell transgenic fish, including A/F Protein, Inc., which has generated transgenic Atlantic salmon that

contain a Chinook salmon growth hormone gene and exhibit enhanced growth, and AquaBounty Farms, which has developed transgenic salmon that grow two to 13 times the size of conventional salmon (Exhibit B, at page 1 of 2).

Similarly, the Virginia Cooperative Extension of the Virginia Tech University reported in April 2002 that, as of January 2002, the FDA was considering petitions to allow the commercial use of transgenic salmon and trout with enhanced growth characteristics, and further reported that researchers were working with transgenic catfish, carp, tilapia, clams, oysters, and abalone (see Exhibit C, at page 3 and 4 of 5). In addition, another article indicated that A/F Protein, Inc., has about 10,000 to 20,000 transgenic salmon, which grow 4-6 times faster than non-transgenic salmon and exhibit 20% improvement in feed conversion (see Exhibit D, at page 1 of 3).

Furthermore, several stable transgenic zebrafish lines exhibiting tissue-specific expression of a fluorescent protein have been generated and are commercially available (see Exhibit E, from Zygogen llc website). As indicated in Exhibit E (at page 1 of 3), the fish lines were generated by injecting embryos with the transgene, with approximately 10% of the embryos expected to demonstrate stable integration of the transgene. As such, it is submitted that it is well known and routine in the art to efficiently generate transgenic aquatic species, including species engineered to exhibit increased growth.

2. Regarding the rejection

Turning to the issues raised in the Office Action, it is stated in the Office Action that the specification discloses generating transgenic aquatic organisms by introducing the transgene into embryonic stem (ES) cells, but that Mullins et al. report that only mouse ES cells have been used to successfully obtain germline transmission of transgenic animals. Applicants point out, however, that the subtitle of the Mullins et al. article is "Trangenesis in the Rat and Larger Mammals". Thus, it would appear that the Mullins et al. reference may not be particularly relevant to the claimed methods of producing transgenic aquatic organisms exhibiting increased muscle mass because there is no

indication in the article that organisms other than mammals were considered (see, for example, paragraph bridging pages S37-S38, referring to mice, rats, sheep and cattle).

Furthermore, it is noted that, except for claim 8, there is no requirement that a transgenic aquatic organism be generated from ES cells. In this respect, it is noted that Mullins et al. state that "microinjection continues to be the method most widely used for other species" (page S37, first paragraph), and, while the specification discloses that ES cells can be used for practicing a method of the invention (page 58, lines 10-19), the specification further discloses that a transgene can be microinjected into embryonal cells from various stages of development, including cells of a zygote, which are the best target for microinjection of the transgene (page 56, lines 13-15), and further discloses that a retroviral vector can be used to introduce the transgene into cells of an embryo, for example, into cells of a blastomere *in vitro* or via intrauterine administration (page 57, lines 14, to page 58, line 8). As such, the specification discloses introducing a transgene into various embryonal cell types, including zygote and blastomere cells, which are well known and routinely used target cells for transgenic procedures.

It is also stated in the Office Action that Moreadith et al. teach that the producing of transgenic knockouts is unpredictable because disruption of a gene may not result in the anticipated phenotype (emphasis in OA, at page 8). There is no indication as to whether the statement is taken from the reference; if the statement is not from the reference, it is unclear why the emphasized term not is modified by "may".

It is stated in the Office Action that Moreadith et al. teach that "gene targeting at a particular locus is unpredictable with respect to the resulting phenotype since often the generation of the knockout animal, in many instances, changes the prevailing notions regarding the functions of the encoded proteins (OA, at page 9, second sentence; emphasis added). Applicants wish to clarify, however, that, in discussing knockout mice, Moreadith et al. refer to "the creation of mutant animals, some of which have unpredictable and subtle phenotypes...." (page 212, first column, first full paragraph; emphasis added). In addition, Moreadith et al. recognize that a knockout method utilizing homologous recombination "allows one to modify precisely the gene of interest" (page 208, second

column, second paragraph; emphasis in original). As such, it is submitted that Moreadith et al. recognize the specificity with which transgenic knockout organisms can be prepared, while acknowledging that, in some cases, an unexpected phenotype may occur.

In support of the alleged unpredictability of a phenotype due to a gene knockout, the Examiner refers to the results obtained when the endothelin gene was disrupted, wherein, instead of detecting abnormal blood pressure, the knockout animals exhibited Hirschsprung's disease (page 208, second column, second paragraph). It would appear from the reference, however, that, prior to the described endothelin gene knockout experiments, the effect of a disruption of the endothelin gene was not previously known because Moreadith et al. state that "if one had even predicted these mice would survive...one might have been in a minority!" (*Id.*; exclamation in original). As such, even if the phenotype due to endothelin gene knockout was 'unpredictable', any prediction of the knockout appears to have been based on no prior knowledge of the effect that endothelin gene disruption may cause in an organism.

Applicants submit that the results of the endothelin gene knockout experiment are readily distinguishable from the facts of the present case because it is well known that disruptions of the GDF-8 gene, including at least two different types of mutations as described by Kambadur et al., McPherron et al., and Grobet et al. resulted in cattle having increased muscle mass, and because knockout of the GDF-8 gene in mice resulted in mice having increased muscle mass (see specification, Example 8, pages 74-77). Thus, even if, in general, and absent any other evidence, it may be unpredictable that disruption of a gene would produce a particular phenotype, it is well known in the art that that various disruptions of the GDF-8 in mammals results in increased muscle mass.

It is alleged in the Office Action that the art is unpredictable as to inducing double muscling in an organism, especially with respect to predicting the phenotype in different species, as indicated, for example, by Kambadur et al., reporting that mutations in the GDF-8 of cattle is associated with muscle fiber hyperplasia, but not hypertrophy, as compared to the subject application, disclosing that

GDF-8 gene knockout in mice is associated with muscle fiber hyperplasia and hypertrophy. However, the claims require that a transgenic aquatic organism exhibits increased muscle mass. As such, Applicants submit that muscle mass is the relevant "phenotype" for consideration, not muscle fiber hyperplasia or hypertrophy. In this respect, Applicants point out that the cattle described by Kambadur et al., McPherron et al., and Grobet et al., and the knockout mice disclosed in the subject application all exhibit the same phenotype, i.e., increased muscle mass.

Applicants further submitted that the distinction of muscle fiber hyperplasia and hypertrophy goes to the mechanism by which increased muscle mass occurs. It is well recognized, however, that an inventor need not understand the mechanism by which an invention works (see Cross v. Iizuka 224 U.S.P.Q 739 (Fed. Cir. 1985), at page 741, note 3). As such, it is submitted that it is irrelevant whether a method of the invention results in increased muscle mass due to muscle fiber hyperplasia, hypertrophy, or both; in view of Kambadur et al., McPherron et al., and Grobet et al., and further in view of the increased muscle mass exhibited by the GDF-8 knockout mice as disclosed in the specification, one skilled in the art clearly reasonably would have predicted that disruption of the GDF-8 gene would result in increased muscle mass.

It is also stated in the Office Action, based on the alleged different effects observed in cattle, as described by Kambadur et al., and in the GDF-8 knockout mice as disclosed in the specification, that "It is certainly less predictable what effects disruption of the GDF-8 gene would have on different species of aquatic animals" (OA, page 9, last full sentence). However, as discussed above, the various disruptions of the GDF-8 gene, including gene knockout by homologous recombination (specification, Example 8) and at least two different types of mutation (see Kambadur et al.), are associated with increased muscle mass in mice and cattle. As such, it is clear that disruption of the GDF-8 gene in mammals predictably results in increased muscle mass and, therefore, that undue experimentation would not have been required for one skilled in the art to practice the claimed invention. Furthermore, as discussed in Section E1, above, transgenic aquatic organisms, including those genetically modified to exhibit increased growth (see, for example, Exhibits B and C), are known in the art. As such, it is submitted that, in view of the specification and of knowledge in the

art, the skilled artisan reasonably would have predicted, for example, that knockout of a GDF-8 gene in an aquatic organism would result in a transgenic organism that exhibits increased muscle mass.

It is further stated that the claims are very broad, being directed, for example, to transgenic crustaceans, mollusks, and chordates (OA, page 10). As discussed above, however, numerous species of transgenic aquatic organisms have been generated and are known in the art, including, for example, transgenic clams and oysters (i.e., pelecypods) and transgenic shrimp (crustaceans) (see, for example, Exhibit C, at page 3 of 5). As such, while the claims are directed to various different transgenic aquatic organisms, such transgenic organisms are known in the art.

In addition, as noted in Section B, above, the basis of this rejection encompasses organisms that were set out as a separate "species" of aquatic animals. As such, it would appear that examination of the subject application includes what are otherwise alleged to be a non-elected species.

It is also stated in the Office Action that the specification provides no working examples of transgenic aquatic organisms, or how to reliably generate such organisms. As discussed in Section E1, above, however, transgenic aquatic organisms, including those exhibiting enhanced growth) are well known in the art. In addition, the methods such as those disclosed in the specification as useful for generating the transgenic aquatic organisms are routine and well known, including, for example, microinjection of a transgene that can undergo homologous recombination (see Moreadith et al., and specification at Example 8). Furthermore, as reported by Zygogen llc (Exhibit E), transgenic zebrafish can be generated with about a 10% efficiency rate. Thus, the evidence of record clearly indicates that undue experimentation would not be required for one skilled in the art to practice the claimed invention.

It is further stated in the Office Action that the specification does not disclose a phenotype for the claimed transgenic aquatic animals. Applicants are uncertain of the basis of this rejection because

the claims clearly require that the transgenic organisms exhibit "increased muscle mass" as compared to a wild type animal.

In summary, it is submitted that transgenic aquatic organisms, including those exhibiting enhanced growth, are well known in the art and, further, that it is well known that various disruptions of GDF-8 genes result in increased muscle mass of an organism. As such, it is submitted that one skilled in the art, viewing the subject application and having knowledge of the art, would have known that transgenic aquatic organisms exhibiting increased muscle growth can be produced by disrupting an endogenous GDF-8 gene using routine methods. Accordingly, it is respectfully requested that the objection to the specification be withdrawn, and that the corresponding rejection of the claims as lacking enablement be removed.

The objection to the specification and corresponding rejection of claims 1 to 15 under 35 U.S.C. § 112, first paragraph, as allegedly lacking a written description are respectfully traversed.

It is stated in the Office Action that the specification does not explicitly define the term "disruption" and, therefore, the term can include disrupting any portion of the GDF-8 gene, as well as disruption of genes that regulate the GDF-8 gene. As an initial matter, it is noted that the claims have been amended such that the term "disruption" is used only with respect to an aspect of the invention that includes insertion of a transgene into the endogenous GDF-8 gene (see claim 1), and new claims directed to transgenes that encode a molecule that "interferes with expression" of GDF-8, e.g., an antisense GDF-8 nucleotide sequence (see claim 64), have been added. It is submitted that, in view of the amendments, it is clear that the term "disrupt" is used according to its commonly understood meaning (e.g., to break apart or interrupt).

Applicants do not disagree that the disruption can include disrupting any portion of the GDF-8 gene, provided the disruption results in the transgenic aquatic organism exhibiting increased muscle mass, and, as evidenced by the disclosure in the specification (Example 8) and, for example,

Kambadur et al., and absent any objective evidence to the contrary, it is submitted that any disruption of an endogenous GDF-8 gene would result in such a transgenic aquatic organism.

It is stated in the Office Action that the term "transgene" can be interpreted to mean any exogenous genetic element (including elements 1 nucleotide in length)" (OA, at page 14). Applicants submit, however, that one skilled in the art would not have understood a transgene to include "elements 1 nucleotide in length." As such, it is requested that this objection be removed or, in the alternative, that the Examiner provide objective evidence indicating that one skilled in the art would consider a single nucleotide to be a "transgene".

It is also stated in the Office Action that the specification does not disclose any species of disruption of a GDF-8 gene in an aquatic organism so as to reasonably convey to one skilled in the art that Applicants' were in possession of the claimed invention at the time the subject application was filed. However, the subject application discloses knockout of a GDF-8 gene in mice and further discloses that GDF-8 polypeptides of aquatic organisms share substantial sequence identity with GDF-8 of mammalian species (see, for example, Figure 3C). In view of this disclosure, it is submitted that the skilled artisan would have known that the genes encoding the various GDF-8 polypeptides also can share substantial sequence identity and, therefore, that knockout of a GDF-8 gene in an aquatic organism can be obtained using methods as disclosed in the specification. The artisan further would have known that methods useful for practicing the claimed invention (e.g., microinjection) are well established and routine.

In summary, in view of the specification, which discloses that GDF-8 gene knockout in mice results in increased muscle mass in the mice, and which further discloses routine and well established methods for introducing a polynucleotide into a cell and for generating a transgenic organism containing such a transgene, it is submitted that the skilled artisan would have known that a GDF-8 gene in an aquatic organism can be disrupted, and that such disruption would result in the organism exhibiting increased muscle mass, and, therefore, that Applicants were in possession of the claimed invention. Accordingly, it is respectfully requested that the objection to the specification be

withdrawn, and that the corresponding rejection of the claims as lacking a written description be removed

The Examiner is invited to contact Applicants' undersigned representative if there are any questions relating to this application. The Commissioner is authorized to charge any additional fees that may be required, or credit any overpayments, to Deposit Acct. No. 50-1355.

Respectfully submitted,

Dated: June 12, 2002

Lisa A. Haile, J.D., Ph.D.
Reg. No. 38,347
Telephone: (858) 677-1456
Facsimile: (858) 677-1465

GRAY CARY WARE & FREIDENRICH LLP
4365 Executive Drive, Suite 1100
San Diego, CA 92121-2133
Customer Number: 28213

Exhibits A through E

EXHIBIT A

MARKED VERSION SHOWING AMENDMENTS

A. In the Specification

The specification was amended as follows:

The invention relates generally to growth differentiation factor-8 (GDF-8[; GDF-8]) and specifically to nucleic acid sequences encoding GDF-8 polypeptide from a variety of aquatic organisms, as well as transgenic aquatic organisms having a disrupted GDF-8 gene and methods of making the same.

B. In the Claims

Claims 1, 4, 8 and 13 were amended as follows:

1. (Amended) A transgenic non-human aquatic organism whose genome comprises a disruption of an endogenous growth differentiation factor-8 (GDF-8) gene, wherein said disruption comprises an insertion of a transgene into the endogenous GDF-8 gene, and wherein said disruption results in said animal exhibiting increased muscle mass as compared to wild-type animal.

4. (Amended) The transgenic aquatic organism of claim 1, wherein the transgene comprises a [GDF-8 antisense polynucleotide] selectable marker sequence.

8. (Amended) A method for producing a transgenic aquatic organism exhibiting an increase in muscle mass, said method comprising:

a) introducing a transgene comprising a selectable marker sequence into [a] an aquatic organism embryonic stem cell;

b) introducing said embryonic stem cell into an aquatic organism embryo;

c) transplanting said embryo into an appropriate pseudopregnant aquatic organism;

d) allowing said embryo to develop to term; and

e) identifying a transgenic aquatic organism whose genome comprises a disruption of [the] an endogenous GDF-8 gene, wherein said disruption results in said aquatic organism exhibiting increased muscle mass as compared to a wild-type aquatic organism.

13. (Amended) The method of claim [8] 7, wherein the transgene comprises a GDF-8 antisense polynucleotide, which interferes with expression of GDF-8.

28 May 2002

Environmental Media Services
a nonprofit organization dedicated to providing journalists with the most current information on environmental issues

Printer-friendly version

Fish

Last update: October, 2000

About 20 varieties of finfish and shellfish have been genetically engineered in the lab, including salmon, catfish, carp, pike, shrimp, striped bass and shellfish. Typically, GE fish are engineered with genes that speed growth, but cold tolerance, disease resistance and flavor enhancement have also been developed. None has yet been approved for commercial use and sale.

In April 2000, the Canadian company A/F Protein, Inc. became the first company to apply to the FDA to sell transgenic fish on supermarket shelves. The company has created GE Atlantic salmon engineered with a growth hormone from Chinook salmon and genes that prevent freezing. Picture of salmon

AquaAdvantage® salmon, developed by AquaBounty Farms, is also awaiting approval by the FDA. The company claims that this salmon can grow two to 13 times the size of conventional salmon and grow to as much as four to six times faster than conventional fish.

The FDA's Center for Veterinary Medicine has responsibility for creating U.S. policy on transgenic fish.

No laws or regulations exist specifically for GE animals, so the FDA regulates transgenic fish under drug laws since the fish includes a GE growth hormone considered a "production" drug for animals.

This process has meant that the FDA review of GE fish has been conducted without public participation. In fact, FDA rules prohibit the agency from revealing what products are under review, so the FDA cannot admit or deny whether an application for approval of any transgenic fish is under way. The pending approval of the GE salmon is known because the companies hoping to market them have made public announcements.

Environmental Risks

The greatest environmental risk from GE fish is from their release into the wild. If transgenic fish escape from their holding pens into the wild - as other farm-raised fish frequently do - they will likely have a competitive advantage over wild fish, many of which are already badly depleted. Crossbreeding between conventional and GE fish is also a concern. (See

CHEMICALS & HEALTH
BIOTECHNOLOGY

Reporters' Guide to Genetic Engineering in Agriculture:
- Table of Contents
- Experts Directory

Fast Facts

Press Releases

Where to Get More Information?

See also:
- Organic Food
- Biosafety Protocol

sidebar)

One approach to meeting these concerns is to engineer sterility into transgenic fish, but achieving 100 percent sterility is not likely. A/F Protein claims to have a technique for sterilization that is 100 percent effective, but a November 1999 article in The New York Times reported that scientists outside the company found that some of A/F's eggs were, in fact, fertile.

Experts - Anne Kapuscinski, Jane Rissler, Rebecca Goldburg, Richard Howard, William Muir

---

Environmental Media Services
1320 18th Street NW 2nd Floor
Washington, DC 20036
(202) 463-6670
Staff Email Directory
Website comments and general reporter inquiries: ems@ems.org

Exhibit C

Virginia Tech

Virginia Cooperative Extension
Knowledge for the Commonwealth

Animal Biotechnology

Authors: Randy Vines, Extension Specialist, Biotechnology Information; Virginia Tech

Publication Number 443-003, April 2002

Table of Contents

Biotechnology in Animal Medicines, Vaccines, and Diagnostics

Biotechnology has yielded new and improved medicines for animals that help lower production costs and improve animal well being by fighting diseases caused by bacteria and parasites. For example, scientists have identified a new anti-bacterial compound that may serve as a substitute for using some antibiotics in animals, a practice that has been criticized for contributing to the increased prevalence of drug-resistant bacteria in human infections.

New or enhanced animal vaccines have also been developed through modern biotechnology techniques. Vaccines are now used to prevent diseases including: foot and mouth disease, scours, brucellosis, shipping fever, feline leukemia, rabies, and infections affecting cultivated fish.

Biotechnology has led to the development of rapid test kits to diagnose the health of livestock and companion animals. Some kits are commercially available, but they will have to be low cost and easy to use if they are to be widely accepted.

Return to Table of Contents

Biotechnology and Transgenic Animals

Advances in scientific discovery and laboratory techniques in the last half of the twentieth century resulted in the ability to manipulate the deoxyribonucleic acid (DNA) of organisms and gave rise to transgenic animals. The use of transgenic animals may accelerate classical breeding programs and provide a means for the economical production of life-saving pharmaceuticals.

To understand how a transgenic animal is produced, it is necessary to review the basic components and functions of living organisms.

Steps used by Virginia Tech researchers to

Genes and the Genome

Animals are made of billions of cells all working together. Every cell of the animal has a complete "instruction manual" or genome (pronounced "JEE-nõm") that is inherited from the parents of the animal as a combination of their genomes. The genome resides in the nucleus of the cell.

Genes are found within the genome and serve as the "words" of the instruction manual. When a cell reads a word (expresses a gene), a specific protein is produced. Proteins give an individual cell, and therefore the animal, its form and function. Genes (words) are written using the four-letter alphabet A, C, G, T. The letters stand for four chemicals called bases, which together compose DNA.

DNA is universal in nature, meaning that the four chemical bases of DNA are the same in all living organisms. Consequently, a gene from one organism can function in another organism.

When a new gene is put into an animal's genome it is said to be transgenic. Scientists have also discovered how to genetically modify (GM) when and/or where in an animal a gene is expressed. For example, it is possible to produce new proteins in the milk of a cow and nowhere else in its body.

Making a Transgenic Animal

One way to produce transgenic animals is through a technique called microinjection. Once scientists have identified and isolated the piece of DNA comprising the gene to be transferred, it is injected into a fertilized egg of the desired animal using a very small glass needle visualized under a microscope. In approximately one percent of the injected eggs, the gene becomes a new "word" in the egg's "instruction manual" by physically combining with the egg's genome. Ideally, the new gene integrates into the genome before the egg begins to divide. If this occurs, every cell in the animal can contain the new protein and the animal will pass the gene on to its offspring. After injection of the gene, the fertilized egg is implanted into a surrogate mother where it fully develops into a transgenic animal.

Traits Being Introduced Into Animals

Currently, the only routine commercial use of transgenic animals (primarily mice) is in the area of human disease research. One way to characterize the range of genetic modifications that are being considered for use in animals is in the three broad areas of input, output, and value-added traits. Examples of each are described below.

Input traits

An "input" trait helps livestock and dairy producers by increasing production efficiency.

Input traits that are being investigated for use in animals:

- Faster, more efficient growth rates
- Increased production of milk or wool
- Resistance to diseases caused by viruses and bacteria

Output traits

An output trait helps consumers or downstream processors by enhancing the quality of the animal product.

Output traits that may prove to be beneficial:

- Leaner, more tender beef and pork
- Milk that lacks allergenic proteins, or results in increased amounts of cheese and yogurt

Value-added traits

By adding or modifying genes, animals can function in completely new ways.

- Producing large amounts of therapeutic proteins in animal milk may be an efficient, relatively low cost method to manufacture many proteins used to treat human diseases or proteins that have industrial value.
- Transplanting animal organs into humans, or xenotransplantation, can be made more successful by genetically modifying the organs so that they are not as readily rejected by the human immune system.
- Development of animals that serve as models for human diseases to help scientists better understand prevention and treatment strategies.

Table 1. Proteins with therapeutic and industrial value that have been produced (but not commercialized) in the milk of transgenic animals include:

Protein	Animal	Use
Antithrombin III	Goat	Reduce the amount of blood needed in some surgeries
Factor VIII, Factor IX	Goat, Pig, Sheep	Treatment of hemophilia
CFTR	Sheep	Treatment of cystic fibrosis
Lactoferrin	Cow	Natural antibiotic and used in coronary surgery
Alpha-1-antitrypsin	Sheep	Treatment of cystic fibrosis and emphysema
Lysostaphin	Cow	An anti-bacterial compound that prevents mastitis in cows
Spider silk protein	Goat	Production of ultra-strong, lightweight medical and industrial materials

Risks and Regulation of Transgenic Animals

One concern of transgenic animal technology is the welfare of the animals. Developmental and health abnormalitites have been reported in conjunction with its use; therefore, researchers must take care to minimize animal suffering.

The inadvertant release or escape of transgenic animals (particularly fish) into the wild where they could breed or compete with the natural population is often cited as a potential risk to the environment. The actual risk associated with this will depend on the type of animal and the nature of the genetic modification; however, where appropriate, procedures must be in place to alleviate this concern.

At the Federal level, the Food and Drug Administration, the Department of Agriculture, and the Environmental Protection Agency are required to regulate transgenic animals and their products to ensure that they are safe for public use and the environment. Depending on the nature of the genetic modification, and the proposed use of the resulting animal or product, more than one agency may be involved in the approval process.

Transgenic fish and shellfish

Aquaculture, worth an estimated $46 billion in 1997, produces approximately one-third of all fish and shellfish consumed by humans. As of January 2002, the FDA was considering petitions to allow the commercial use of transgenic salmon and trout with enhanced growth characteristics. In addition, researchers are working with transgenic catfish, carp, tilapia, striped bass, clams, oysters, shrimp, and abalone.

Traits being tested in transgenic fish include:

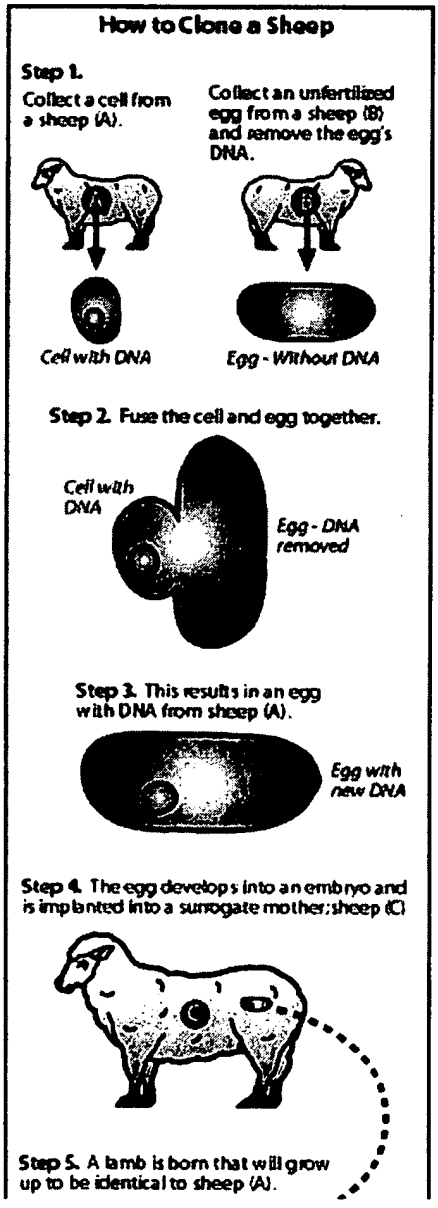
- Growth rates that are 3-11 times faster with more efficient feed utilization
- Increased tolerance to cold water
- Improved disease resistance

Safety and risk issues associated with transgenic fish:
Issue: Introduction of a protein that is potentially allergenic or toxic to humans
Solution: Extensively test before the FDA grants market approval
Issue: Escape of a transgenic fish into the wild, leading to interbreeding and/or competition with the natural population.
Solution: Restrict cultivation to land-locked facilities or guarantee sterility of transgenic species

Return to Table of Contents

Biotechnology and Cloning Animals

Advances in biotechnology have allowed scientists to make genetically identical copies or clones of animals. Duplication of an organism's genome occurs naturally when identical twins are born or when a plant is grown from a cutting of another plant. However, the world really took notice of cloning in 1997 when a group of Scottish researchers announced the birth of Dolly the sheep, which had been cloned using a single cell from an adult sheep. Dolly had only one "parent;" her nuclear genome was exactly like her "mother's" instead of being a combination of two parents. Therefore, Dolly could generally be thought of as her mother's identical twin.



To produce Dolly, scientists took an egg from a sheep and removed its nucleus (which contains the genome or instruction manual), rendering it unable to function or develop. Next, they took a cell with an intact genome from a different adult sheep (Dolly's "mother") and fused it to the sheep egg which lacked a genome. The egg, with its new genome, was stimulated to begin developing into an embryo and was implanted into a surrogate sheep where it grew normally, resulting in the birth of Dolly. Dolly later gave birth to normal lambs.

Benefits and Risks of Cloning
Researchers have cloned other mammals including cows, goats, pigs, and mice. However, the overall low rate of successful cloning and frequent occurrence of developmental abnormalities in cloned animals demonstrate the need for further research before cloning will be practical.

It has also been reported that cloned animals may exhibit health problems throughout their life. Cloned animals may age prematurely as Dolly was diagnosed with arthritis at a seemingly young age and cloned mice had a shorter than normal life span. Additionally, it was demonstrated that cloned mice were both larger in size and heavier than a control group of non-cloned mice.

If advances in animal cloning technology were to overcome the current obstacles, the most obvious benefit would be the ability of a farmer to have a herd of superior performing animals in one generation. Breeding companies could sell cloned embryos in a manner similar to the way in which semen is currently marketed. A potential drawback of this practice would be the loss of genetic diversity in livestock herds, but this could be avoided by limiting the number of cloned embryos of a given animal that were sold.

It has also been proposed that cloning could be used to increase the population of animals in an endangered species. The mouflon sheep, which is a wild Mediterranean sheep with less than 1000 animals remaining, was successfully cloned. Additionally, scientists are attempting to clone an endangered wild Asian ox, called the guar (the first cloned guar died of an intestinal illness shortly after birth) and possibly the giant Panda. Although possible, a recovering population of cloned animals would be hindered by a lack of genetic diversity and would not address the larger issue of how the animal became endangered.

Return to Table of Contents

View this document in PDF format

Visit Virginia Cooperative Extension

Exhibit D

COMMERCIALIZATION OF PATH-BREAKING TRANSGENIC SALMON FACES STUMBLING BLOCKS

Transgenic Atlantic salmon could soon be the first commercially produced food product derived from a genetically modified animal. Transgenic Atlantic salmon produced by A/F Protein Inc. (Waltham, MA) reportedly grow up to four to six times faster than non-transgenic salmon and exhibit a greater than 20% improvement in feed conversion efficiency(1). The company has 10,000 to 20,000 transgenic salmon in indoor tanks at three facilities in the Canadian Maritime provinces. A/F hopes that these fish will become the broodstock for producing eggs for commercial aquaculture in Canada, New Zealand, Chile, and the United States. However, commercialization of this path-breaking product faces a number of stumbling blocks. Against the background of both favorable and unfavorable reports in the popular media, the anticipation of a key regulatory decision, and actions against production of transgenics by certain salmon producers, the commercialization of transgenic salmon is proving contentious.

Commercialization of transgenic salmon in the United States will depend upon regulatory approval by the Food and Drug Administration (FDA), which must approve the marketing of any products derived from animal biotechnology. An FDA decision on approval of the A/F transgenic salmon is expected in the near future. The FDA Center for Veterinary Medicine is regulating the transgenic salmon expressing an introduced growth hormone gene as a new animal drug(2). That is, transgenesis is being regarded as a means for delivering growth hormone to the tissues of the fish. Hence, regulatory approval of the A/F salmon will depend on rigorous demonstration that the transgenic salmon are safe to eat.

Approval of marketing transgenic salmon would constitute a "significant federal action" posing impacts to the environment. Under the National Environmental Policy Act, FDA must consider biosafety issues posed by commercial production of the transgenic salmon. Ecological concerns include competition of transgenic stocks with wild populations, introgression of the transgene into wild gene pools, heightened predation of transgenics on prey populations, and a range of other possible impacts. Because ecological concerns are site-specific, it may prove necessary to control the sites where transgenic fish are reared, as well as the level of confinement in production facilities(2). Regulatory authorities may require that production stocks be sterile triploids or all-female triploids. Any level of confinement other than absolute containment in indoor, recirculating aquaculture systems will have to be assessed for specific sites. Decision support tools have been developed to assess and manage any risks posed by research and development activities with genetically modified fish and shellfish(3) and by larger-scale production and marketing of genetically modified organisms(4).

Commercialization of transgenic fish also faces issues of consumer and

commercial acceptance. A number of salmon producers groups feel that growing public distrust of genetically modified foods can create a potential marketing problem for the salmon industry. The industry already faces heightened public scrutiny because of controversies regarding possible environmental impacts of ocean net-pen aquaculture of salmon. Against this background, certain salmon producers or producers groups have distanced themselves from production of transgenic salmon. On February 25, King Salmon of New Zealand announced that it had killed all of its transgenic chinook salmon and disposed of them in accordance with containment protocols. The action came days after New Zealand environmentalists had convinced the government to conduct a review of the licensing and inspection process for the experiments. In a unanimous vote of its Board of Directors on February 24, the British Columbia Salmon Farmers Association adopted a ban on use of transgenics by its members. In 1996, the Scottish Salmon Association distanced itself from experiments with transgenics carried out by Otter Ferry Salmon.

Elliot Entis, CEO of A/F Protein Inc., feels that environmental concerns can be addressed by producing transgenic salmon in closed aquaculture systems or by producing sterile fish, and consumer concerns by showing that there are no food safety issues to hide. The company hopes to gain FDA approval and to begin commercial production and marketing of its fish by 2001.

Sources

1. Fletcher GL, Shears, MA, Goddard SV, Alderson R, Chin-Dixon EA, and Hew CL. 1999. Transgenic fish for sustainable aquaculture. In Sustainable aquaculture: Food for the future?, eds. Svennevig N, Reinertsen H, and New M, 193-201. Rotterdam: AA Balkema.

2. Matheson J. 1999. Will transgenic fish be the first ag-biotech food producing animals? FDA Veterinarian XIV (III), http://www.fda.gov/cvm/fda/infores/fdavet/1999/may.htm

3. Performance standards for safely conducting research with genetically modified fish and shellfish. http://www.isb.vt.edu/perfstands/psmain.html 1997.

4. Scientists' Working Group on Biosafety. Manual for assessing ecological and human health effects of genetically engineered organisms. http://www.Edmonds-Institute.org 1998.

Eric M. Hallerman
Department of Fisheries
and Wildlife Sciences
Virginia Tech
mailto: ehallerm@vt.edu

Overview Home

Technology

Z-Tag™ - Proprietary Transgenic Zebrafish Technology

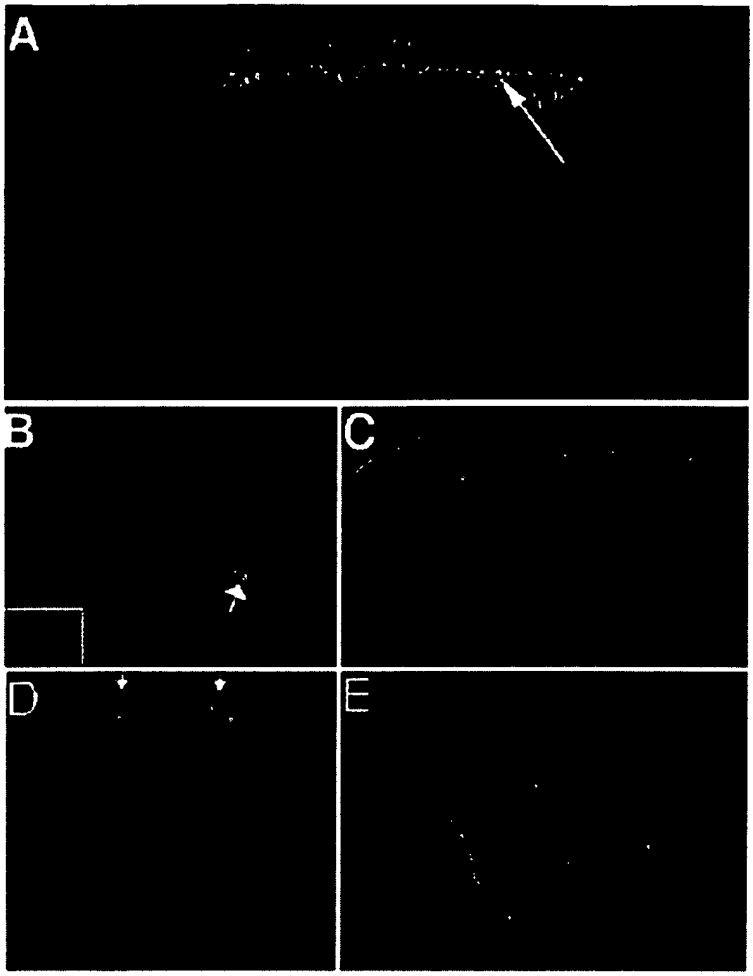
Zygogen utilizes its transgenic zebrafish technology, Z-Tag™, in three ways:

- Development of disease models for secondary screening/compound profiling
- Functional genomics
- Drug target discovery

Zygogen has several stable transgenic fish lines that show tissue-specific expression of a fluorescent protein. Each fish line is designed by injecting several hundred embryos with a DNA construct containing a unique tissue-specific zebrafish promoter fused to a GFP gene. Approximately 10% of these embryos will stably integrate the construct. In order to determine germ-line integration, these embryos are raised to sexual maturity (3 months) and their offspring are screened for tissue-specific expression of the fluorescent protein. Once established, these lines have been demonstrated to retain their initial fluorescence over years.

Zygogen Co-Founder, Dr. Shuo Lin, developed Zygogen's proprietary technology. The patented, "Transgenic Fish with Tissue-Specific Expression" is a comprehensive composition of matter and method patent that applies to zebrafish with tissue-specific expression of a gene.

To date Zygogen has transgenic lines that fluoresce in the blood, neurons, thymus, blood vessel and olfactory system. Zygogen scientists are now designing several additional fish lines that will express a fluorescent marker in tissues such as the liver, muscle, bone, pancreas, heart or specific subsets of neurons. The entire panel of fluorescent fish lines will be used to screen for gene and small molecule function. Under development is a multi-fluorescent organ fish to streamline this analysis. Zygogen designs custom fish lines in response to specific client needs.



A. Blood **B.** Thymus **C.** Central Nervous System
D. Olfactory Neurons **E.** Motor Neurons

Disease Model Development
Secondary Screening/Compound Profiling

Zygogen is designing zebrafish disease models that can be used to screen libraries of small molecules and gene products. Therapeutic areas of focus are those in which zebrafish provide a significant advantage over mice, including **angiogenesis, neurodegeneration** and **cardiovascular**.

Zygogen is developing high throughput processes that can be used in conjunction with the disease models. Zebrafish embryos can live for several days in 96-well plates and small molecules/gene products can be added to these wells with reproducible effects. By using Zygogen's disease models in a high throughput system, preclinical research is accelerated directly to the drug candidate identification stage, skipping over the target identification/validation step of drug discovery. The zebrafish disease model can then be used iteratively to optimize drug candidates for both function and toxicity.

Functional Genomics

Zebrafish Tools
The zebrafish genome will be sequenced by the Sanger Center over the next couple of years, with data expected to be released starting fall 2001. Zygogen uses the most recently developed molecular and bioinformatics tools to leverage zebrafish for functional analysis of genes. Assays can be designed to look at all stages of and, by virtue of working with a whole-organism, viability and general morphology can be checked in parallel. Zygogen customizes solutions for partners that integrate the latest advancements in tool development.

Bioinformatics
Zygogen is creating a proprietary zebrafish genetic database, Z-Base™, which contains public and private zebrafish sequence data. Zygogen has entered into a strategic partnership with NuTec Sciences for the development of the database. NuTec Sciences has created algorithms for Baylor University and TGIR to enable the Human Genome Project. NuTec Sciences recently announced a partnership with IBM to build the nation's second largest supercomputer (the largest privately-owned). It will be located in NuTec's new worldwide headquarters in Atlanta, Georgia and Zygogen will have direct access to the supercomputer.

New Target Discovery

Isolation of novel tissue-specific genes
Zygogen is identifying novel, tissue-specific genes in zebrafish that are highly conserved between zebrafish and humans. Transgenic zebrafish embryos expressing a fluorescent protein in specific cell lineages under the control of a tissue specific promoter provide a unique opportunity to purify the earliest embryonic progenitor cells from different tissues. Tissue-specific cDNA libraries have been constructed using both surgery and fluorescence activated cell sorting (FACS) to isolate the specific cell lineages labeled in the transgenic fish lines. Zygogen expects to identify hundreds of novel genes with a tissue-specific expression pattern.

The gene function is further analyzed by over-expression or blocking expression in transgenic zebrafish models. For example, a novel TNF (tumor necrosis factor) receptor has been discovered and shown to alter red blood cell production in transgenic zebrafish. Human orthologues of the zebrafish proteins will be identified using sequence alignment from either public or private databases.

Secreted and cell membrane-bound proteins are known to have important roles in cell-cell interactions, cell proliferation and survival, and tissue and organ morphogenesis. Such proteins can be considered potential drug targets or therapeutic molecules. Zygogen can provide significant value to the pharmaceutical/biotechnology industry by identifying novel secreted proteins and more importantly, by providing a functional assay for these proteins in different tissue types.